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Some Functional Properties of Protein Isolates from Yeast, *Saccharomyces fragilis*

Pong Vananuvat and John E. Kinsella*

Functional properties of protein isolates prepared from the yeast *Saccharomyces fragilis*, by alkaline and water extraction and precipitated by acid (pH 4.0) and by heating (80°) at pH 6.0, respectively, were examined. Typical solubility curves were obtained with maxima occurring above pH 7.5. Minimum solubility points occurred at pH 4 to 4.5 and 4.5 to 5 for alkali and water extracts, respectively. Heat precipitation reduced protein solubility by approximately 5% compared to acid precipitation (pH 4 at 26°). The foaming capacity of yeast proteins, with the exception of the water extract precipitated at pH 4, was inferior (about 50%) to that obtained with soy isolate. Foam stability of all yeast proteins was poor. The emulsifying activity of all yeast proteins, except the sample prepared by heat precipitation of alkaline extract at pH 6.0, had higher emulsifying activity than the soy isolate. Protein prepared from water extract and precipitated at pH 4.0 showed the highest value. Yeast protein isolates exhibited lower surface tension than water.

In the last decade intensive research has been carried out to find cheaper sources of protein to alleviate protein malnutrition and new sources of functional proteins for food industry. Proteins from fish, oilseed, leaves, and microbes have been intensively investigated and some new proteins are available commercially for human consumption. Higher production rates and protein yields, ease of production control, and possible food production without the use of limited land makes single-cell protein (protein derived from cells of yeast, mold, bacteria, and algae) more attractive as a protein source compared to conventional plant and animal sources (Mateles and Tannenbaum, 1968). However, a number of problems associated with single-cell protein (SCP) have to be solved to render it a safe and cheap source of protein for human use. Proteins from microbial cells should be low in cell wall fragments to improve nutritional value, i.e. bioavailability of protein. Nucleic acid content should be reduced to minimize the intake of nucleic acid to less than 2 g per day (Edozien et al., 1970) and the protein should have acceptable color, flavor, and texture (McCormick, 1973). These criteria are fundamental and necessitate the isolation of protein from yeast cells prior to its use in foods. Initially the utility and marketability of isolated yeast protein will depend to a large degree on their functional properties, cost notwithstanding. Information on the functional properties of proteins prepared from microbial cells is limited (Labuza et al., 1972). Rheological properties were investigated by Huang and Rha (1971). Fiber formation was studied by Huang and Rha (1972) and Mitsuda et al. (1971).

In this paper some functional properties of proteins isolated from *Saccharomyces fragilis* were determined.

Department of Food Science, Cornell University, Ithaca, New York 14853.

EXPERIMENTAL SECTION

Saccharomyces fragilis was grown in continuous culture on crude lactose as reported (Vananuvat and Kinsella, 1975a). Final cell concentration was about 11 g/l. Recovery of yeast and extraction of protein were performed as described (Vananuvat and Kinsella, 1975c). Protein was extracted from the broken cells with 0.4% sodium hydroxide or water. Protein was precipitated from these respective extracts by acidification with 1 *N* HCl to pH 4.0 and by heating the extracts to 80° for 30 sec following adjustment of the extract to pH 6.0. The precipitated protein was recovered by centrifugation (10,000*g* for 15 min) and freeze dried. Thus, four types of yeast protein isolates were tested for functionality. Protein samples 1 and 2 were prepared by extracting the broken cells with NaOH and precipitating the protein with acid at pH 4.0 (26°) and by heat (80°) at pH 6.0, respectively; samples 3 and 4 were prepared by extracting with water and precipitating the protein under identical conditions.

Solubility. Solubility of yeast protein isolates (1% concentration) was determined according to Lu and Kinsella (1972). Kjeldahl nitrogen was determined by the method of AOAC (1965) and protein was calculated using a factor of 6.25. Protein was also determined according to the method of Lowry et al. (1951) for comparison. Bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) was used to prepare the standard curve.

Whippability. Whipping capacity was determined according to the method of Yasumatsu et al. (1972) where foam expansion and foam stability were taken as indices of whippability. Fifty milliliters of protein suspension (0.1 g/ml) in a 100-ml stoppered cylinder was shaken horizontally for 1 min. The resulting foam volume (milliliters) was defined as foam expansion. The residual foam volume, measured after 30 min, was used as an index of foam stability.

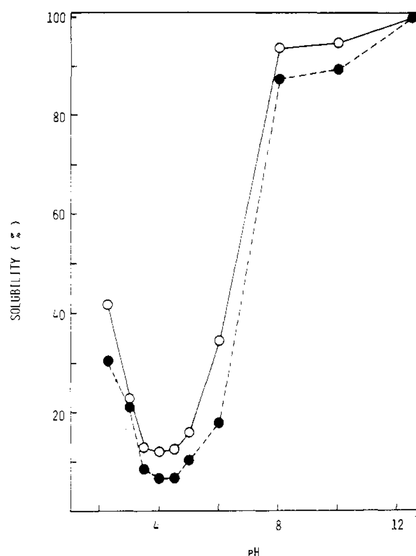

Figure 1: Solubility profile of protein isolated from *S. fragilis*. Protein was extracted from *S. fragilis* with 0.4% sodium hydroxide and precipitated at pH 4.0 (sample 1): (O) Kjeldahl nitrogen × 6.25; (●) protein determined by the method of Lowry et al. (1951).

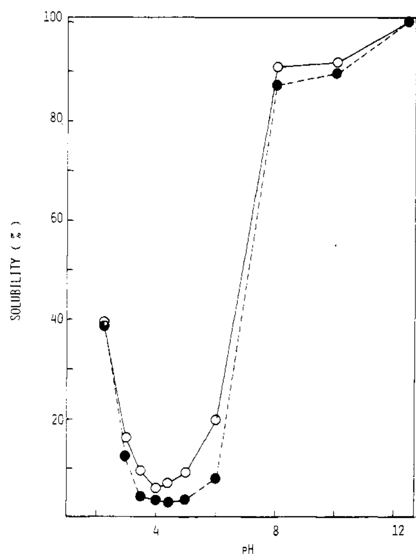

Figure 2. Solubility profile of protein isolated from *S. fragilis*. Protein was extracted from *S. fragilis* with 0.4% sodium hydroxide and precipitated by heat (80°) at pH 6 (sample 2): (O) Kjeldahl nitrogen × 6.25; (●) protein determined by the method of Lowry et al. (1951).

Emulsifying Activity. Emulsifying activity was measured according to Yasumatsu et al. (1972). To a protein suspension (7%) in water (pH 7.0) 25 ml of vegetable oil was added and the mixture was emulsified in a Waring Blendor (semi-micro jar) at maximum speed for 1 min. The resultant emulsion was equally divided into two 50-ml centrifuge tubes (20 ml capacity) and centrifuged at 1300*g* for 5 min at 20°. The height of the emulsified layer divided by the total height of the mixture multiplied by 100 was taken as an index of emulsifying activity.

Surface Tension. Surface tension was determined using a 5% yeast protein solution (pH 7.3) with a Roller-Smith balance equipped with a Wilhelmy Plate Tensiometer (Biolar Corp., Northgrafton, Mass.). Surface tension (dynes/centimeter) was calculated from the formula $(F \times 0.98)/W$, where F corresponds to the tensiometer reading (milligrams) and W indicates the perimeter of the blade (5 cm).

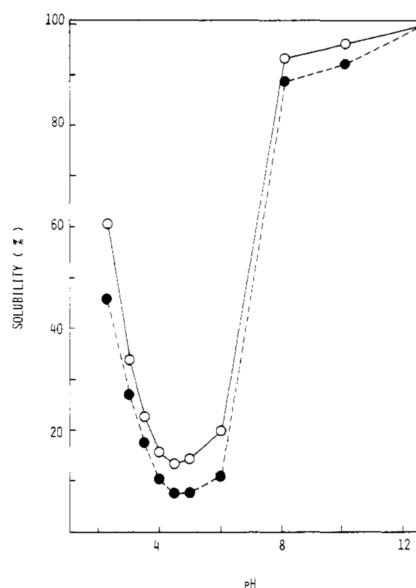

Figure 3. Solubility profile of protein isolated from *S. fragilis*. Protein was extracted from *S. fragilis* with water and precipitated at pH 4.0 (sample 3): (O) Kjeldahl nitrogen × 6.25; (●) protein determined by the method of Lowry et al. (1951).

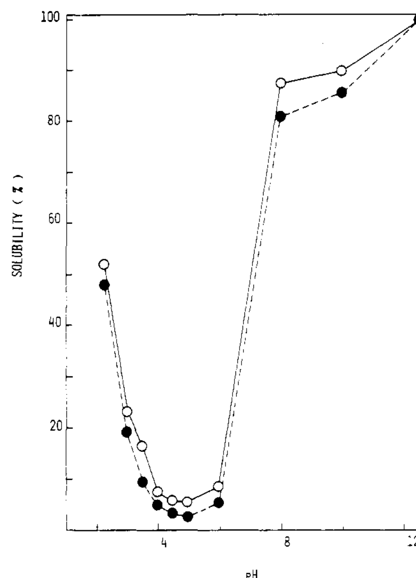

Figure 4. Solubility profile of protein isolate from *S. fragilis*. Protein was extracted from *S. fragilis* with water and precipitated by heat (80°) at pH 6 (sample 4): (O) Kjeldahl nitrogen × 6.25; (●) protein determined by the method of Lowry et al. (1951).

RESULTS

The solubility profiles of yeast protein prepared by the different methods were quite similar (Figures 1–4). At each pH studied, the solubility of the protein as determined by the Lowry method (true protein) was less than that indicated by the micro-Kjeldahl method (total soluble nitrogen) and these differences were larger near the isoelectric points. A sharp increase in the solubility occurred at pH values between 6 and 8 when about 85% of the proteins were solubilized.

The minimum solubility range occurred between pH 4 and 4.5 for protein prepared from NaOH extracts (i.e., samples 1 and 2) whereas the minimum solubility for protein prepared from water extracts was around pH 4.5 and 5.0 (samples 3 and 4). The minimum solubility of protein precipitated by heat (samples 2 and 4) was lower than that

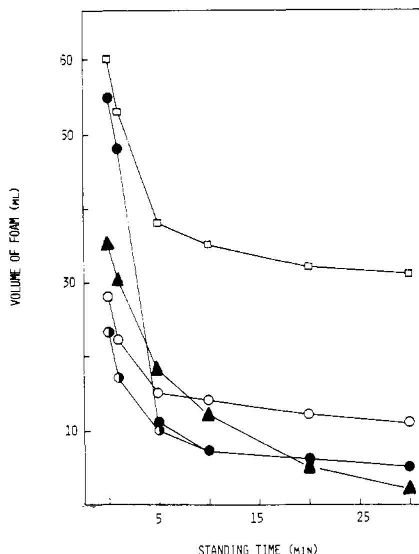

Figure 5. A comparison of the whipping properties of protein isolates prepared from *S. fragilis* with those of soy isolate. The height of foam at zero time and at 30 min indicated foam expansion and foam stability, respectively: (▲) protein from *S. fragilis* extracted with 0.4% NaOH and precipitated at pH 4.0 (sample 1); (O) protein from *S. fragilis* extracted with 0.4% NaOH and precipitated at pH 6.0 with heat (sample 2); (●) protein from *S. fragilis* extracted with water and precipitated at pH 4.0 (sample 3); (◑) protein from *S. fragilis* extracted with water and precipitated at pH 6.0 with heat (sample 4); (□) soy isolate.

obtained by acid precipitation (samples 1 and 3). There was a sharp increase in solubility of protein extracted with water (samples 3 and 4) at pH below the minimum solubility range.

The whipping property of yeast protein isolates was evaluated in a single system by the method of Yasumatsu et al. (1972). Soy isolate (Supro 350, Ralston Purina Co., St. Louis, Mo.) was used for comparison. The foam expansion of protein prepared from sample 3 showed the highest value and was quite comparable to soy isolate (Figure 5). Sample 4 gave the lowest foam expansion. Although the foam expansion of sample 3 was high the foam volume decreased sharply after 1 min. The foam stability, defined as the residual foam volume after standing 30 min, was approximately the same for three types of protein isolates whereas sample 2 showed a higher value. In contrast to yeast protein, the foam volume of soy isolate was more stable. The foam produced by whipping the solution of yeast protein isolates resembled soy isolate in their physical appearance and texture.

The emulsifying activity of the four types of yeast protein isolates was measured and compared with the soy isolate. All yeast proteins except sample 2 each had a higher emulsifying activity than soy isolate (Table I). Sample 2 had a negligible emulsifying activity.

The surface tension of solutions of protein isolates from *S. fragilis* was studied and compared with those of solutions of soy isolate and water (Table I). All solutions of yeast protein had lower surface tension than water. Sample 3 had the lowest value, i.e. average 55.6 dyne/cm. Soy isolate demonstrated lower surface tension than all of the yeast protein isolates studied.

DISCUSSION

An important functional property of protein isolates is their solubility at various pH values and in addition to serving as a useful indicator of protein denaturation, the solubility profile may also suggest possible uses especially in beverages (Samson et al., 1971; Mattil, 1971). Proteins should be soluble under the conditions of pH, ionic

Table I. Comparison of Emulsifying Activity and Surface Tension of Solutions of Proteins from *Saccharomyces fragilis* with Soy Isolate

Protein[a] samples	Emulsifying act.[b]		Surface tension, dyn/cm[b]	
	Av	Range	Av	Range
1	51	48–59	57	54–59
2			56	53–57
3	67	61–70	55	51–57
4	59	57–63	56	54–58
Soy isolate	46	41–53	53	48–55

[a] Samples 1 through 4 were described in Figure 5. [b] As defined in the Experimental Section. Surface tension of water was 70.

strength, etc., at which the protein will be used, especially in beverages. Protein isolates from *S. fragilis* showed good solubility at pH 2 and pH values above 7. The minimal solubility of yeast protein at pH ranging from 3 to 6 may limit its use in many foods in solubilized form. However, it can be dispersed in solid food systems. Solubility of some proteins can be improved by the formation of sodium or calcium proteinate derivatives (Lu and Kinsella, 1972; Mattil, 1971; Meyer, 1966), and by enzymic modification (Cheftel et al., 1971).

The solubility profiles of *S. fragilis* protein isolates are very similar to those obtained with proteins from glandless cottonseed meal, sunflower seed meal, and coconut meal (Lawhon and Cater, 1971), alfalfa meal (Lu and Kinsella, 1972), soybean leaf (Betschart and Kinsella, 1973), soybean meal (Circle, 1950), and peanut meal (Fontaine and Burnett, 1944). The minimum solubility occurs at pH values between 4 and 5. However, the proteins from *S. fragilis* yeast, cottonseed meal, and sunflower meal were substantially less soluble at low pH than are those of coconut meal, soybean meal, peanut meal, alfalfa leaf, and soybean leaf. Although high pH improves the solubility of all protein mentioned, it may induce extensive racemization of amino acids (Tannenbaum, 1968; Tannenbaum et al., 1970). Thus the alkaline process of protein solubilization should be more thoroughly investigated before it is applied industrially.

The minimum solubility range of protein prepared by alkaline extraction of *S. fragilis* was 4.0 to 4.5, whereas that obtained by water extraction was 4.5 to 5.0, i.e. different types of extractant caused a shift of the isoelectric point of the protein. The pI is a function of the nature and concentration of solutes present in the protein solution (Mahler and Cordes, 1966). Heat precipitation decreased the solubility of yeast protein isolates by about 3 to 5% in the case of both water and alkaline extracts. Heating may rupture hydrogen bonds, affect salt bridges or hydrophobic interactions, and oxidize disulfide bonds (Schultz and Anglemier, 1964) and thus yield protein possessing different conformation and properties, i.e., less soluble than the native protein.

The foam expansion of yeast protein isolates from water extract precipitated at pH 4.0 is comparable to soy isolate but the foam stability of these yeast proteins was much lower. The whipping properties of yeast protein isolates precipitated with heat were inferior to the samples prepared without heat. Similar results were reported for whey protein where heat treatment was detrimental to the whipping quality and decreased both air uptake and foam stability (Hansen and Black, 1972). The same authors suggested that heat denaturation of the protein might be contributing to the decrease in foaming ability. Garibaldi et al. (1968) reported that damage to the whipping property of

egg white was due to heat denaturation of the ovomucin-lysozyme complex. In general, foam persistence is the result of the interaction of several factors, including surface tension, viscosity, temperature, pH, ionic strength, and concentration of protein in solution (Briskey, 1968; Hansen and Black, 1972).

All yeast protein isolates except those prepared by heat precipitation at pH 6.0 from alkaline extracts showed good emulsifying activity and were slightly superior than soy isolate. The yeast protein isolates prepared from water extracts and precipitated with heat possessed lower emulsifying activity than the samples precipitated without heat. Lawhon and Cater (1971) also found that some functional properties of protein isolates from glandless cottonseed processed with heat were inferior to those of isolates from unheated meal.

The yeast proteins lowered the surface tension of aqueous solutions. However, they were not as effective as soy isolates in this respect. This property was reflected in the lower foam stability of the yeast protein isolates compared to soy isolate.

Yeast protein isolates, especially those obtained by water extraction (Vananuvat and Kinsella, 1975b, 1975c) which are low in nucleic acids and have a good amino acid balance, should have potential commercial application in meat emulsions, ground meats, and bakery goods. These isolates possess a light creamy color, little flavor, and good emulsifying properties.

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Physicochemical Properties of Peanut Flour as Affected by Proteolysis

Larry R. Beuchat,* John P. Cherry, and Michael R. Quinn[1]

Defatted peanut flour was hydrolyzed with pepsin, bromelain, and trypsin. Nitrogen solubility was increased substantially in water at pH 4.0–5.0 and in 0.03 *M* Ca^{2+} at pH 4.0–11.0. Water adsorption by the flour when exposed to various relative humidities was increased as a result of hydrolysis. Emulsion capacities in water and in 0.5 *M* NaCl were completely destroyed during digestion and water- and oil-retaining properties were reduced when compared to control samples. Gel electrophoretic patterns showed substantial qualitative changes in enzyme-treated peanut protein. Patterns were different for each of the hydrolysis treatments.

Physicochemical properties of defatted peanut flour depend upon naturally occurring characteristics associated with the peanut kernel as well as processing conditions to which the kernel is exposed during conversion to flour. Behavioral properties of peanut proteins in the presence of carbohydrates, fat, water, and other food ingredients are of greatest interest, since protein comprises approximately 60% of defatted flour. Peanut flours represent potentially valuable ingredients in the formulation of protein-fortified food products.

Modification of vegetable and animal proteins to improve particular functional requirements in food systems has attracted considerable research attention. Moist and dry heat treatments (McWatters and Heaton, 1974; Neucere et al., 1969; Neucere, 1972), acid hydrolysis (Better and Davidsohn, 1958; Fontaine et al., 1946; Higgins et al., 1941), fungal fermentation (Beuchat et al., 1975; Quinn and Beuchat, 1975), and frozen storage in the presence and absence of reducing agents (Cherry and Ory, 1973) were found to have marked effects on the physicochemical properties of both peanuts and peanut flours. Proteolytic enzymes were reported to improve functional properties of proteins from cottonseed (Arzu et al., 1972), soybean (Roozen and Pilnik, 1973), rapeseed (Hermansson et al., 1974), whey (Kuehler and Stine, 1974), and egg (Grunden et al., 1974). This paper describes the effect of enzymatic hydrol-

Department of Food Science, University of Georgia Agricultural Experiment Station, Experiment, Georgia 30212.
[1] Present address: Department of Nutrition, Rutgers University, New Brunswick, New Jersey 08903.